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Levels of Detoxifying Enzyme (General Esterases and GST) in the Worker and the Soldier Castes of *Odontotermes obesus* (R.): A Possible Adaptation to Tolerate Exposure to Pesticides

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Abstract

North Bengal Tea Plantations produces about 24% of Indian tea. Using synthetic pesticides to an extent of 5.799 to 9.793 Kg $l^{-1}ha^{-1}yr^{-1}$ has resulted in selection of more tolerant pest populations. Among the tea pests, termite occupies a distinct niche by attacking tea plants from under soil or at collar region of a bush. Termites are important subterranean pests in tea where they limit the establishment of newly planted young tea and reduce the mature tea by attacking their frame and killing bushes. Termites are social insects with a highly elaborate system of labor division. Based on the labour division in the colony, it has been observed that worker caste is more exposed to the toxic xenobiotic or pesticide residues of soil and organic matter of tea plantations, than any other castes including soldier castes of a colony. A comparative study of detoxifying enzymes General Esterases (GE) and Glutathione S-Transferase (GST) between worker and soldier castes of *Odontotermes obesus,* a major subterranean pest of tea, was conducted. Quantitative analysis of GE showed a 3.46 folds higher expression in worker termites compared to that of the soldiers. GST was also 2.45 folds more in workers than in soldiers. The differential feeding and foraging habits of the workers of *O. obesus* may explain the observed differences with the soldiers in these detoxifying enzymes. As the workers search for food, digest them and also feed the other castes their contact and exposure to pesticide residue in soil and organic debris is much which possibly explains the more level of detoxifying enzymes in them for a greater tolerance to these xenobiotics for survival in the pesticide changed soil system of tea plantations than soldier or reproductive castes.

1. Introduction

Tea is a plantation crop growing under monoculture practice. Great demand of this foliage crop in domestic and international market prompted a wide spread tea cultivation in an area of 1,07, 479 ha in sub-Himalayan region of North Bengal, India. The total made tea produced yearly in North Bengal is about 200 million kg representing about 24% of total Indian production (Anonymous, 2003). Tea as perennial crop is time to time attacked by 1031 species of arthropod pests across the world, of which only 300 species are recorded in India and about 167 species from North east India (Das, 1965; Muraleedharan, 1992; Rattan, 1992; Sivapalan, 1999). These pests cause 11 to 55% loss in yield (Gurusubramanian et al., 2008). To protect the tea crop against pest attack, organosynthetic pesticides are commonly applied (5.799 to 9.793 kg $l^{-1}ha^{-1}yr^{-1}$) in this region. This application is a burden to planters as well as to environment and can result in a resurgence of primary pests (Sivapalan, 1999) or cause mite syndrome and secondary pest outbreaks (Cranham, 1966), development of resistance (Kawai, 1997; Sivapalan, 1999) and environmental contamination, including presence of undesirable residues on made tea (Chaudhury, 1999; Sivapalan, 1999).

Among the pests, termites are more surreptitious and damaging because of its subterranean existence. There are approximately 2700 termite species in the world, of which 269 are recorded from Indian subcontinent (Kapur 1962; Kambhampati and Eggleton 2000; Su and Scheffrahn, 1986). Among these 183 species are known to damage buildings and 83 species cause significant damage amounting to several millions of rupees annually (Edwards and Mill, 1986; Kapur and Bose, 1972). Termites cause damages not only to agricultural crops, forests and stored-products but also to structural timber and buildings. Kapur (1958) and Das (1962) reported termite as a serious menace to Tea industries causing an estimated loss of at least 15% of the tea crop due to the wood eating termite in North-Eastern India. Das (1962), Sands (1977) also reported widespread termite damage to tea bushes in most of the tea gardens in Darrang and Cachar where 50 to 100% bushes may be affected. The species *Odontotermes assamensis* Holmgren, *O. parvidens* Holmgren & Holmgren and some others of the genera such as *Odontotermes* and *Microcerotermes* are also reported from tea gardens in India.

Termites live in a colony where each caste is being assigned with a specific function. The queen and the king are there in the colony to produce fertilized egg (Korb, 2008). The alates are winged reproductives preparing to swarm out of nest, then pair and to start new colonies (Eggleton, 2011). Soldiers' only work is to defend colony and to escort foraging workers (Traniello, 1981; Kaib 1990; Rupf and Roisin 2008). Whereas, workers are the mainstay, they forage food and water, build and repair colony structures, and tend the immatures, alates, soldiers, the king and the queen (Eggleton, 2011). The labour division in the colony, and the nature of work compels the worker caste to get the maximum exposure to the toxic xenobiotic or pesticides [*viz.* Cypermethrin (Synthetic Pyrethroid), Chlorpyriphos (Organophosphate), Carbaryl (Carbamate) and Lindane (Organochlorine) etc.] more than any other castes in a colony (Bordereau and Pasteels, 2011). Evidences suggest that elevated activity of enzymes like esterases, monoxygenases or glutathione s-transferases, helps insect to tolerate the toxicity of xenobiotics (Motoyama and Dauterman, 1980; Oppenoorth et al., 1979; Kostaropoulos et al., 2001; Valles and Yu, 1996). The present study was undertaken to understand the levels and status of two of the defense enzymes, general esterase and glutathione S-transferase, in two of the major castes, the workers and the soldiers of *Odontotermes obesus* collected from tea plantation the. An attempt has also been made to relate the defense enzyme level in these castes with their activity pattern in the colony.

2. Materials and Methods

2.1. Termite collections

Termites were collected from the tea plantations of North Bengal. Mounds (termitaria) were located and then fungal gardens were extracted from the mounds. Fungal gardens are maintained by worker castes and defended by the soldiers, hence with location of fungal garden finding these castes were easy. The collected samples were maintained at constant temperature of 25±2°C and Relative Humidity of 75-80%. The termites were identified as the subterranean termite, *Odontotermes obesus,* based on key characteristics of the soldiers (Chhotani, 1997).

2.2. General esterase activity

General esterase activity was measured by using α-naphthyl acetate (α NA) as substrate according to the method of van Asperen (1962) with few modifications. 20 µl of supernatant was taken in each well of the 96-well microplate reader in duplicate. 200 µl α NA (30 mM) was added to each well for reaction. The reaction was stopped after 10 minutes by adding 50 µl of staining solution containing 0.1% Fast BB salt and 5% SDS (2:5). The plate was left for five minutes for equilibration and absorbance was recorded at 590 nm in a microplate reader (DYNEX MRX_{TC} *Revelation*). The change in absorbance was converted to end product formation from a standard curve of α-naphthol (5-500 nM). Blanks were set at the same time using reaction mixture without protein extracts.

2.3. Glutathione S-transferase (GST) activity

The activity of GST was measured using 1-chloro-2, 4-dinitrobenzene (CDNB) and reduced glutathione (GSH) in conjugation reaction as described by Habig et al. (1974). The reaction mixture contained 150 µl of GSH and 50 µl CDNB. The enzyme solution (30 µl) was then added to the above mixture. The content was shaken gently and incubated at 25^0C for 2-3 minutes. A blank was run concurrently in the reference slot of the spectrophotometer. Absorbance was recorded for 6-7 minutes at 340 nm. The increase in absorbance over 5 minutes period was considered for calculation.

2.4. Esterase native PAGE electrophoresis

Esterase native Polyacrylamide Gel Electrophoresis (PAGE) was done in a BIOTECH vertical electrophoresis unit by using a 10.0% polyacrylamide separating gel (8×7 cm), 5% stacking gel (2 cm), and tris-glycine buffer. Each sample (20 *µl* of protein) was loaded with 10% glycerol (wt:vol) and diluted 1:1 with running buffer. Bromophenol blue (0.1%) was used as a marker dye. Electrophoresis was conducted at a constant 120 V. The gels were stained for esterase activity in 100 ml of 0.1 M phosphate buffer (pH 6.5) containing 2% α-naphthyl acetate and 0.04 g fast blue BB salt at 25°C for 1 h (Prabhakaran and Kamble, 1993).

3. Results and Discussion

General Esterase (GE) and Glutathione S-Transferases (GST) were estimated in worker and soldier castes of *O. obesus* collected from a conventional (pesticide treated) tea plantation. Quantitative analysis of GE showed higher expression in worker termites compared to that of the soldiers. A 3.46 folds increase in GE, 2.45 folds increase in GST was observed, respectively (Table 1). Densitometric analysis of the electrophoretic gels showed an expression of GE both in worker and Soldier castes of *O. obesus.* In workers higher levels of expression of the GE was evident through a dense gel band than that of a soldier castes (Figure 1). While, several bands were expressed for GE isozymes in workers, soldiers did not express any prominent band. It may be interpreted that discrete activity and foraging habits of workers and soldiers of *O. obesus* may be the cause of their differential expression. The polymorphism in the termite colony shows the physiological range of the esterases in different castes which are supposedly involved in detoxification processes and/or in the hydrolysis of juvenile hormone (JH) (Terriere, 1984). Controlling termite pest is difficult because of their subterranean nature. Planters often apply the pesticides at the affected bushes and termitarium to control termites. Besides regular exposure of the workers to lethal xenobiotics, pesticide residues also cause stress to these soil handlers as long as the toxic molecules exist in the plantation soil. As a consequence of the constant exposure to xenobiotics the termites, especially the worker caste seems to have evolved effective defense mechanisms to deal with these stresses (Scott, 1995). According to the report by Bishnu et al. (2009), North Bengal tea ecosystem contains higher residue level of pesticides like these of chlorpyrifos and heptachlor than the prescribed MRL. Studies of Scharf et al. (2003, 2005), suggest that workers have higher levels of expression of genes involved in breaking down cellulose. So the differential feeding and foraging habits of the workers of *O. obesus* and their consequent exposure to environmental xenobiotics or pesticide residues may explain the observed differences with the soldiers for the detoxifying enzymes. Enhanced levels of the two detoxifying enzymes in workers possibly endows them with a greater tolerance to the xenobiotics and pesticide-contaminated soil of tea plantation, hence providing better survival value to the chemically-stressed worker caste.

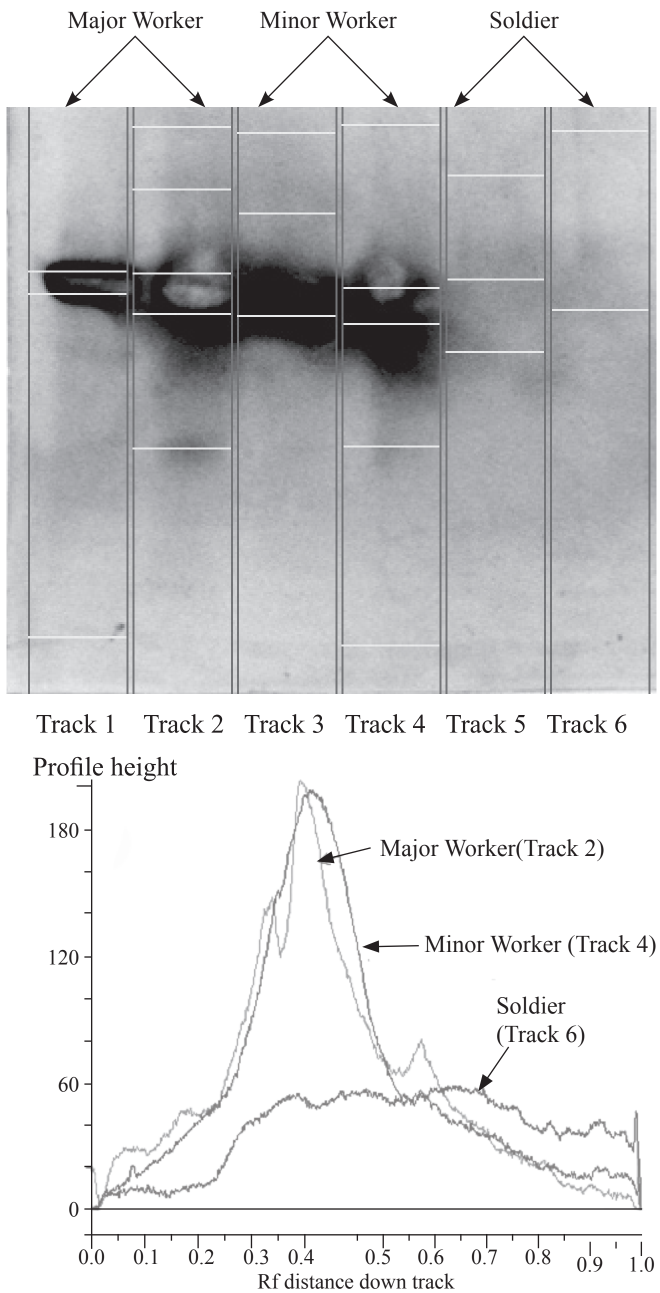

Figure 1: Densitometric analysis of electrophoregram of general esterases of *Odontotermes obesus* from Worker and Soldier castes

Table 1: Quantity of defense enzymes (GE and GST) of worker and soldier castes of *Odontoterems obesus*

Tea Plantation	GST (μM min^{-1}mg^{-1} protein) (Mean*±SD)	General Esterase (mM mg^{-1} protein) (Mean*±SD)
Worker	299.747^a±80.284	9.436^a±5.00
Soldier	122.336^b±14.658	2.726^b±2.41

*Different alphabets in the columns denote significant differences at 0.05% level of probability

4. Conclusion

Differential expression of these detoxifying enzymes highlighting the difference at physiological level between worker and soldier caste of *O. obesus* may be due to differential feeding and foraging habits of castes in a termitarium.

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